Introduction: Fibrocartilaginous metaplasia in tendons is considered a functional adaptation to compressive loads and/or stress shielding (lack of tensile loads) [1,2]. However, chondral metaplasia was seen in calcific insertion Achilles tendinopathy [3] and cartilage-specific markers were upregulated in a rat supraspinatus overuse model [4]. Excessive or inappropriate fibrocartilaginous matrix production may therefore be part of the pathological process in tendinopathy. We studied whether chondrogenic differentiation was present in mid-portion Achilles tendinopathies. We hypothesize that chondrogenic differentiation might be used as target for treatment of tendinotic lesions. Therefore we aimed at developing an in-vitro model of chondrogenic tendon differentiation for future use in evaluating treatment opportunities for tendinopathy.

Materials and Methods: After informed consent, peroperatively harvested biopsies of chronic mid-portion Achilles tendinopathic lesions (n=12) and of macroscopically healthy Achilles tendons (n=5) were analysed by microscopy and by real-time RT-PCR for the following chondrogenic differentiation markers: (sex-determining region Y)-box9 (SOX9), aggrecan (AGC1), collagen 2 (COL2A1), RUNT-related transcription factor 2 (RUNX2), collagen 10 (COL10A1).

For culture studies, non-degenerative hamstring tendon tissue (n=7 adolescents) and non-degenerative Achilles tendon tissue (n=2 adults) were harvested (approved by the local medical ethical committee). Explants were cultured 21 days in chondrogenic medium (DMEM; ITS; TGFβ2 10 ng/ml; vitamin C 0.1 mM) or control medium (DMEM; 10% FCS). Samples were harvested on days 7, 14, 21 for gene expression analysis. In separate experiments, on day 14 either triamcinolone (10–4 M) or platelet-rich plasma (PRP, 20% vol/vol) were added to modulate the chondrogenic differentiation. Samples were harvested on day 21.

Statistical analysis was performed using a Kruskall-Wallis H test (only in the culture studies) and post-hoc Mann–Whitney U test with significance set at p<0.05.

Results: Mid-portion Achilles tendinotic lesions had significantly increased glycosaminoglycan staining and more rounded cell nuclei than healthy tendons. Gene expression of chondrogenic differentiation markers SOX9, AGC1, COL2A1, RUNX2, but not collagen 10 were significantly upregulated (Figure 1). Non-degenerative tendon explants cultured on chondrogenic medium for 7, 14, and 21 days had significantly higher expression of AGC1, COL2A1, and COL10A1, but not SOX9 and RUNX2. Removing the chondrogenic stimulus at day 14 reduced expression of AGC1, COL2A1, and COL10A1 at day 21. Adding triamcinolone (10–4 M) from day 14 on reduced COL2A1 expression, whereas other genes were not affected (Figure 2). PRP (20% vol/vol) also appeared to influence chondrogenic expression pattern. (not shown)

Discussion: This study shows chondrogenic differentiation on transcriptional level in mid-portion Achilles tendinopathy. Early and late chondrogenic markers were upregulated in affected tissue, but hypertrophic cartilage formation (collagen 10 upregulation) was not seen in vivo.

In the in-vitro model simulating chondrogenic tendon differentiation, the early chondrogenic marker SOX9 was not upregulated, whereas hypertrophic marker collagen 10 was upregulated. This suggests that the model in its current state may slightly overstimulate the cells towards a hypertrophic chondrogenic lineage compared to the in-vivo situation. However, even in the stage of hypertrophic differentiation, all upregulated chondrogenic markers could still be significantly downregulated by removing the chondrogenic stimulus.

Molecular and/or mechanical signals that influence chondrogenic differentiation may help tenocytes to return to their normal tendon matrix production and may therefore be key factors to improve treatment opportunities for developing tendinopathies. We showed that chondrogenic differentiation of tendon cells could be modulated by addition of a biochemical signal like triamcinolone or PRP in the presence of the chondrogenic environment. The model can therefore be used to test substances for their potentially therapeutic role in controlling chondrogenic differentiation in tendinopathy.